Fatty Acid Composition of *Sterculia* Seeds and Oils from Madagascar

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The percentage contents of oil and protein in the seeds of 17 species belonging to 9 genera of the Sterculiaceae family, growing in Madagascar, were determined. The major fatty acids were palmitic (11.6–27.5%), oleic (8.3–32.6%), linoleic (4.2–45.8%), malvalic (tr–53.7%), and sterculic (1.3–24.8%) acids. *Heritiera littoralis* seed oil was characterized by a high content of malvalic (53.7%) and sterculic acids (12.4%). Cyclopropene fatty acids were identified and determined by using a combination of gas chromatographic and ^1H and ^{13}C NMR analyses. Dihydrosterculic acid was also detected in all samples at a low concentration.

INTRODUCTION

Cyclopropenoid fatty acids (CPEFA) such as malvalic (8,9-methylene-8-heptadecenoic, 18:CE) and sterculic (9,10-methylene-9-heptadecenoic, 19:CE) acids, are found in many plant families, e.g., Bombacaceae, Malvaceae, Sapindaceae, Sterculiaceae, and their natural occurrence has been reviewed by several authors (Smith, 1970; Christie, 1970; Badami and Patil, 1981). More recently, a survey of the Sarcolaenaceae family for CPEFA has been used for plant classification (Gaydou and Ramanoelina, 1983).

Many seed lipids containing CPEFA are extensively consumed by humans in tropical areas (Bohannon and Kleiman, 1978; Ralaimanarivo et al., 1982). Since CPEFA are responsible for some physiological disorders (Phelps et al., 1965), the carcinogenic and synergistic activities of these compounds have been examined on laboratory animals (Lee et al., 1971). Pawlowski et al. (1985) observed that methyl sterculate showed significantly higher activity than methyl malvalate. On the other hand, Schmid and Patterson (1988), studying the effects of CPEFA on fungal growth, discussed the possibility that these compounds serve as protective agents for plants.

The Sterculiaceae family is well represented in tropical areas and particularly in Madagascar since 18 genera and about 280 species are located on this island (Arenes, 1959). Some *Sterculia* species have been surveyed recently for CPEFA content (Patil and Sastry, 1988; Sundar Rao et al., 1989; Sundar Rao, 1991).

This paper contains the first report on the fatty acid composition of 17 species native to Madagascar, some of which are consumed by the local population or cultivated as ornamentals. Since the dihydromalvalic (8,9-methyleneoctadecanoic, DHM) methyl ester peak was not resolved from the vaccenic (18:1ω7) methyl ester peak on Carbowax 20M columns (Bianchini et al., 1981), the quantitative determination of each of these two fatty acid methyl esters (FAME) was not achieved. Nevertheless, as previously observed, small amounts of DHM are detected in the Sterculiaceae family. The contents in DHM are generally much lower than those of DHS in this family (Bohannon and Kleiman, 1978; Vickery, 1981).

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MATERIALS AND METHODS

Materials. Seeds were collected from the plants themselves, in eastern Madagascar (Arenes, 1959) by officers of the Département Recherches Forestières, Ambatobe, Antananarivo, and the authors. The identification of the seeds was done by the Département Recherches Forestières.

Standard Methods. Moisture, oil, ash, and protein contents were determined according to AFNOR Norms (Association Française de Normalisation, 1988).

Halphen Color Test on Oils. The original method (Halphen, 1897) was used for characterization of CPEFA. Equal volumes (about 1–3 mL) of oil, 1-pentanol, and carbon disulfide containing 1% sulfur were placed in a test tube and warmed on a steam bath for 10–15 min. All of the samples gave the characteristic red-pink color.

Preparation of Methyl Esters. Fatty acid methyl esters (FAME) were prepared from oils by base-catalyzed transmethylation with sodium methoxide as previously indicated (Bianchini et al., 1981).

Gas Chromatography (GC). A Delsi 300 gas chromatograph equipped with a flame ionization detector (FID) was used for FAME separation with a fused silica capillary column (50 m × 0.32 mm i.d.) coated with Carbowax 20M (phase thickness 0.15 μm). Column temperature was 170 °C, and detector and inlet temperatures were 200 °C. Helium was used as carrier gas at a inner pressure of 0.7 bar (2 mL min^{-1}). The injections averaged 1 μL of a 2% solution of FAME in hexane. Peaks of the usual fatty acids were identified by comparison of retention times with those of commercially available FAME. CPEFA methyl esters were identified by comparison with those of authentic CPEFA found in kapok seeds (Gaydou et al., 1983). Percentages of FAME were calculated from peak areas given by the electronic integrator.

Nuclear Magnetic Resonance (NMR). ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-400. Samples were prepared in a 5 mm o.d. tube by mixing the oils in a mixture of $CCl_4/CDCl_3$ (90:10 v/v) in a volume ratio 1:20 according to the method of Pawlowski (1972). Tetramethylsilane (TMS) was used as internal standard. The high-field region (0.5–1.5 ppm) of ^1H NMR spectra showed a singlet peak at 0.8 ppm due to the two hydrogens on the cyclopropene ring and a triplet at 1.0 ppm due to the terminal methyl groups of all fatty acids.

RESULTS AND DISCUSSION

The various species of Sterculiaceae investigated and the characteristics of their seeds are given in Table I. The oil content ranged from 1.2 to 42.3%. For sample 9 (hull of *Hildegardia erythrosiphon*), the oil content was very low in comparison with the corresponding nut (1.4 vs 36.1%, respectively), results in agreement with most of the seeds. The oil content of the other hull species was too low for quantitative determination. For some *Nesogordonia* species (samples 11, 13, 15, and 16), the oil content

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Table I. Characteristics of the Sterculiaceae Seeds from Madagascar

sample	genus	species	%[a] moisture	oil	ash	protein
1	*Brachychiton*	*gregorii* F. Muell.	6.7	42.3	4.1	23.5
2	*Dombeya*	*spectabilis* Bojer	5.9	10.0	3.2	16.8
3	*Helmiopsiella*	*madagascariensis* J. Ar.	2.3	35.9	4.6	25.2
4	*Helmiopsis*	*inversa* H. Perr.	4.2	6.5	4.2	22.3
5	*Helmiopsis*	*richardii* (H. Bn) R. Cap.	3.8	12.0	5.1	21.8
6	*Heritiera*	*littoralis* F. Muell.	9.7	8.3	4.3	24.4
7	*Hildegardia*	*erythrosiphon* subsp. *erythrosiphon* var. Ahalamerensis (J. Ar.) J. Ar.	5.4	14.4	3.8	17.6
8	*Hildegardia*	*erythrosiphon* (H. Bn) Kost[b]	5.8	36.1	4.1	15.3
9	*Hildegardia*	*erythrosiphon* (H. Bn) Kost[c]	1.7	1.4	10.2	8.7
10	*Hildegardia*	*perrieri* (Hochr.) J. Ar.	5.3	34.2	4.3	18.4
11	*Nesogordonia*	*crassipes* (H. Bn) R. Cap.	1.9	2.7	3.8	11.7
12	*Nesogordonia*	*macrophylla* J. Ar.	3.3	14.7	4.5	22.3
13	*Nesogordonia*	*normandii* R. Cap.	2.5	4.4	4.2	18.5
14	*Nesogordonia*	*pachynema* R. Cap.	2.4	22.4	4.3	15.3
15	*Nesogordonia*	*routak* R. Cap.	3.1	2.1	3.8	23.5
16	*Nesogordonia*	*thouarsii* (H. Bn) R. Cap.	2.3	1.2	4.1	25.2
17	*Pterygota*	*perrieri* Hochr.	3.4	30.2	4.9	15.7
18	*Sterculia*	*tavia* H. Bn	8.7	14.8	5.1	17.5

[a] Dry basis, mean of two determinations. [b] Nut. [c] Hull.

Table II. Fatty Acid Composition of the Sterculiaceae Seed Oils from Madagascar

fatty acid[a]	species[b] 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
saturated																		
12:0			tr		0.1	tr		0.5	0.7									
14:0	0.1	0.2	3.3	tr	0.3	0.1	0.3	1.3	1.9	0.3	0.0	0.4	1.6	0.3	0.2	0.6	0.2	0.2
16:0	22.1	16.3	18.5	28.3	22.7	11.6	26.0	26.1	23.5	24.8	27.5	19.6	28.1	25.5	18.5	13.4	23.0	27.3
18:0	2.8	0.6	2.1	5.2	1.6	3.0	2.5	1.3	1.9	1.3	2.9	2.3	2.0	2.6	2.8	4.6	2.6	4.7
20:0			0.2		0.2				3.0			0.7	3.0	0.2	0.5			0.5
22:0										2.3				1.1				
unsaturated																		
16:1	0.3	0.4	0.4	0.8	0.3	0.2	0.6	1.3	2.1	0.7	2.5	0.7	3.3	0.6	0.5	0.6	0.8	0.5
17:1	0.8	0.3	0.6		0.9	1.2		0.3	0.3		0.8	2.6		0.4	0.9	2.3	tr	0.2
17:2								4.4	1.3									
18:1ω9	19.9	16.2	24.3	28.7	20.7	8.3	32.6	17.0	21.1	24.3	23.3	19.6	16.1	13.1	8.5	6.1	22.7	13.8
18:2ω6	43.8	45.8	31.8	23.6	37.4	4.2	14.6	23.6	30.3	31.0	25.7	20.6	21.6	20.8	5.7	8.6	28.8	15.8
18:3ω3	0.3	3.3	0.9	2.1	1.2	1.0	0.8	1.1	0.9	1.0	1.1	0.9	2.5	1.1	2.0	2.5	2.5	5.4
cyclopropanoic and cyclopropenoic																		
DHM[c] + 18:1ω7	2.1	1.5	1.6	2.3	1.4	0.9	1.9	1.0	0.8	1.1	0.6	2.0	1.8	2.2	5.5	1.5	1.9	2.3
DHS[d]	0.3	2.0	0.4	2.9	0.5	1.2	1.1	0.5	0.8	0.6	0.6	0.6	1.9	2.0	2.8	3.2	0.3	2.9
18:CE[e]	6.5	7.0	6.4	tr	9.5	53.7	0.3	3.7	1.4	0.9	2.8	19.3	8.7	18.8	23.9	29.9	8.5	7.2
19:CE[f]	1.0	5.7	2.6	3.5	2.3	12.4	19.1	12.1	5.0	4.1	5.4	6.3	3.2	5.0	24.8	20.8	3.4	19.2
others[g]		0.7	6.9	2.6	0.9	2.2	0.2	5.8	5.0	7.6	6.8	4.4	5.4	6.3	3.4	5.9	5.3	

[a] Percent by weight of total fatty acids. [b] For species identification see Table I. [c] Dihydromalvalic acid: 8,9-methyleneheptadecanoic acid. The 18:CA and vaccenic (18:1ω7) methyl esters were not resolved on the Carbowax 20M column used. [d] Dihydrosterculic acid: 9,10-methyleneoctadecanoic acid. [e] Malvalic acid: 8,9-methylene-8-heptadecenoic acid. [f] Sterculic acid: 9,10-methylene-9-octadecenoic acid. [g] Unidentified minor fatty acids.

was poor (1.2–4.4%). All of the oils gave a positive Halphen test showing the presence of CPEFA (Halphen, 1897). Therefore, we chose transmethylation of the oils in methanol using sodium methoxide as catalyst since CPEFA are unstable compounds in acidic medium. Among the various gas chromatographic methods used for CPEFA analyses (Fischer and Schuller, 1980; Park and Rhee, 1988), we chose the determination without further derivatization using a Carbowax 20M column at 170 °C, a method already in use in our laboratory (Bianchini et al., 1981). The thermal stability of CPEFA on the column used was monitored with a sample of kapok FAME analyzed at various temperatures between 150 and 200 °C. Results obtained were compared with ^{1}H NMR analyses (Pawlowski et al., 1972). The CPEFA recovered was 9.6% at 170 °C (10.3% by ^{1}H NMR). These results are in agreement with those previously observed for baobab seed oils (Ralaimanarivo et al., 1982).

The fatty acid compositions of the 17 species are given in Table II. Five fatty acids were found in high amounts in the oils: palmitic (11.6–27.5%), oleic (8.3–32.6%), linoleic (4.2–45.8%), malvalic (tr–53.7%), and sterculic (1.3–24.8%) acids.

Heritiera littoralis seed oil was remarkable by its high content in CPEFA (>66%), content in the same order found for *Sterculia foetida*, 50–72% (Badami and Patil, 1981). To our knowledge, *H. littoralis* is the first species containing such a high amount of malvalic acid (53.7%). These seed lipids constitute an interesting source of malvalic acid for analytic purposes, since *Pterospermum acerifolium* was found to contain 32.2% of this acid (Bohannon and Kleiman, 1978). The content in CPEFA of the *Heritiera* genus show a rather large variation of these fatty acids considering *H. littoralis*, >66%, and *H. actinophylla*, 2.8% (Vickery, 1980).

The chemical shifts of the ^{13}C NMR spectrum of *H. littoralis* oil are given in Figure 1 for the 105–115 ppm region. The different peaks could be unambiguously assigned to the corresponding CPEFA, as indicated in Table III, taking into account the GC percentages. Using

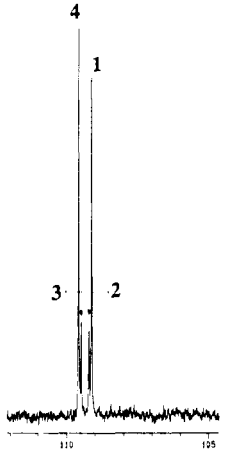

Figure 1. ^{13}C NMR spectrum of the olefinic carbon atoms of *H. littoralis* seed oil. For peak identification see Table III.

Table III. ^{13}C NMR Chemical Shifts of the Olefinic Carbon Atoms of Malvalic Acid (18:CE) and Sterculic Acid (19:CE) in *H. littoralis* Seed Oil

signal no.[a]	CPEFA carbon	chemical shift,[b] ppm
1	18:CE (C8)[c]	109.13
2	19:CE (C9)	109.23
3	19:CE (C10)	109.49
4	18:CE (C9)	109.59

[a] Signal numbers are taken from Figure 1. [b] Expressed against TMS [solvent: mixture of $CCl_4/CDCl_3$ (90:10, v/v)]. [c] Refers to the number of the molecule.

our previous method for the determination of petroselinic acid by ^{13}C NMR spectroscopy (Mallet et al., 1990), we found that the 18:CE/19:CE ratio was 4.1 from ^{13}C NMR, in agreement with the GC ratio (4.33 from Table II).

The *Nesogordonia* genus shows a variable CPEFA content. *N. routak* and *N. thouarsii* have malvalic acid concentrations slightly higher than those of *N. macrophylla* and *N. pachynema* (23.9 and 29.9 vs 19.3 and 18.8%), but they have sterculic acid concentrations much higher than *N. macrophylla* and *N. pachynema* (24.8 and 20.8 vs 6.3 and 5.0%). Meanwhile, *N. crassipes* and *N. normandii* are rather low in both 18:CE and 19:CE. In the *Sterculia* genus, 19:CE content is usually greater than that of 18:CE (Badami and Patil, 1981), an observation confirmed in this work with *S. tavia*.

Small amounts of dihydrosterculic acid (9,10-methyleneoctadecanoic, DHS) occurred in all species investigated. Similar findings were observed in the Sterculiaceae family (Bohannon and Kleiman, 1978; Vickery, 1980).

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